protein) that has a chimeric nucleic acid binding domain and a transcriptional regulatory domain. The chimeric nucleic acid binding domain includes at least two nucleic acid binding motifs, at least one of which is a zinc finger, and the transcriptional regulatory protein has a different binding specificity than does a protein having only one of the motifs. Support for these new claims can be found throughout the specification. To give but a few examples of such support, Applicant directs the Examiner's attention, for instance, to page 5, lines 11-21; page 7, lines 3-17; page 9, line 34-page 10, line 21; etc. No new matter has been added by way of the present Amendment.

As required, attached hereto as **Appendix A** is a marked-up version of the changes made to the claims by the present Amendment. For the Examiner's convenience, also attached hereto is an **Appendix B** showing all pending claims as amended remaining in this application.

Response to Claim Objections and Rejections:

The cancellation of all previously-pending claims renders moot the outstanding objections and rejections in this case. Nonetheless, Applicant has evaluated all of the arguments and art presented by the Examiner, and offers the following remarks evidencing the patentability of the present claims in light of these arguments and art:

All of the pending claims recite nucleic acids encoding transcriptional regulatory proteins with chimeric nucleic acid binding domains that include a zinc finger. None of the references or arguments collected by the Examiner could render such claims unpatentable.

Of the references cited by the Examiner, only Barbas et al., Desjarlais et al. and Ladner et al. describe proteins containing a zinc finger.

Barbas et al.

Barbas et al. is not prior art to the present claims. The effective 102(e) date of Barbas et al., is December 30, 1996 (as published on the first page). The present application was filed more than two years prior to this date (i.e., on December 29, 1994); the presently-pending claims are fully supported by the specification as filed and are entitled to the benefit of this filing date. Accordingly, Barbas et al. is not available as prior art.

Desjarlais et al.

Desjarlais et al. performed footprinting studies with three different proteins, each of which consists of three artificial zinc fingers that have been derived from a consensus zinc finger framework. The first protein consists of three identical zinc fingers. The second protein consists of three zinc fingers that are identical at all but one to four positions within the recognition region. The third protein is a permuted version of the second protein. Desjarlais et al. show that each of these proteins binds to a site in DNA that is a composite of the three zinc finger recognition subsites. Desjarlais et al. point out that the three proteins bind their respective composite sites with widely varying affinities and specificities. Desjarlais et al. further question the quantitative modularity of zinc fingers.

The teachings of Desjarlais et al. cannot anticipate the claimed invention. The proteins studies by Desjarlais et al. lack any transcriptional regulatory domain and do not regulate transcription. Furthermore, the binding affinity of the tested proteins proved unreliable, prompting Desjarlais et al. to question the generality of chimeric DNA binding proteins. There is therefore no teaching or suggestion in Desjarlais et al. that zinc finger motifs could be utilized in chimeric nucleic acid binding domains to produce effective transcriptional regulatory proteins.

Ladner et al.

The Examiner's arguments in the Office Action regarding the teachings of Ladner et al. actually refer to two different Ladner et al. references, that will be discussed separately here. The Examiner first refers to columns 12 and 13 of U.S. Patent No. 5,198,346 (the '346 patent). This section of the patent describes earlier work by Ladner et al. that was reported in PCT Application WO 88/06601 ("the '601 application"). The Examiner's later points refer to the '346 patent itself.

The '601 application describes a system for generating helix-turn-helix DNA binding proteins with new binding specificities. The purpose of the work described in the '601 application is to develop proteins that can act as transcriptional repressors because they bind to DNA and sterically interfere with binding by transcriptional activators (see throughout, including

for example, page 13). The system is designed to imitate the transcriptional regulatory network of bacteriophage lambda, and utilizes lambdoid repressors. These repressors bind to DNA via a helix-turn-helix motif in which one 9-amino-acid motif lies in the major groove of the DNA and makes specific contacts with the bases (see, for example, page 21, lines 23-25). Lambdoid repressors bind as homodimers, and recognize dyad symmetric sites in the DNA. In the '601 application, Ladner et al. describe preparation of a *pseudo*homodimer protein, in which two identical helix-turn-helix DNA binding domains are linked together in a single polypeptide. One of the recognition helices is then mutated, and the mutant polypeptides are screened to identify ones that bind to non-symmetric sites in the DNA. The '601 application itself predicts that the strategy will only rarely produce proteins that bind to DNA, estimating a success rate of only 1 in 10^6.

The teachings of the '601 application are limited to helix-turn-helix DNA binding domains, and to polypeptides that do not affirmatively regulate transcription. This application cannot anticipate the present claims. Moreover, the application cannot render obvious the presently claimed invention. The '601 application's teachings cannot be extended beyond polypeptides that bind to DNA via helix-turn-helix motifs, and specifically cannot be extended to polypeptides that bind to DNA via a zinc finger.

The application mentions the possibility of using some kind of DNA binding motif other than a helix-turn-helix at only one point (page 22, line 8-11), but acknowledges that it would first be necessary to establish that altered specificity versions of the binding motif. Given the limited success predicted in establishing altered specificity helices in helix-turn-helix proteins, this suggestion could not provide any reasonable expectation of success for non-helix-turn-helix proteins. In particular, it cannot render obvious proteins that bind to DNA via a zinc finger. Zinc finger proteins generally do not homodimerize. By contrast, the proteins studied in the '601 application were all homodimerizing proteins, and the application remarks on the improved affinity afforded by dimerization. Even with this "extra" binding help, the '601 application reports poor results in engineering chimeric DNA binding domains. The '601 application therefore *teaches away* from the development of proteins that bind to DNA via any motif other than a helix-turn-helix.

Similarly, the teachings of the '601 application cannot be extended to encompass proteins that include a transcriptional regulatory domain. The polypeptides described in the '601 application do not themselves regulate transcription. In fact, the application notes that proteins that *do* regulate transcription may be utilized in the production of novel specificity binding proteins, so long as they are first mutated so that they no longer interact with RNA polymerase (see, for example, page 22, lines 21-23). In other words, according to the '601 application, proteins are only useful if their ability to regulate transcription directly is destroyed. For this reason, as well as those presented above, the '601 application *teaches away from* the presently claimed invention.

The Ladner et al. '346 patent also cannot anticipate or render obvious the presently-claimed invention. The '346 patent represents improvements and expansions of the ideas and strategies of the '601 application, including applicability to other classes of DNA binding motifs (e.g., zinc fingers in particular, see for example, column 64, line 43 - column 66, line 2). At least some of the improvements render the teachings of the '346 patent, when taken as a whole, even *less* relevant to the presently claimed invention than are the teachings of the '601 application. Such improvements include, for example, *not linking the two DNA binding domains in a single polypeptide*. According to the '346 patent, individual DNA binding domains should preferably be prepared as *separate* polypeptides that either are chemically cross-linked to one another (analogous to Park et al.; see, for example, column 42, lines 39-43 and 52-54), or be allowed to naturally dimerize with one another (see, for example, column 42, lines 26-51 and column 42, line 55 - column 43, line 9, see also Examples 1 and 2). The '346 patent, like the other references, lacks any teaching of a protein that has both a transcriptional regulatory domain and a chimeric DNA binding domain including at least two DNA binding motifs, one of which is a zinc finger.

For all of these reasons, the Ladner et al. reference, taken alone or in combination with other references, can neither anticipate nor render obvious the presently claimed invention.

Conclusion

If it is believed that a telephone conversation would help expedite prosecution of this

case, or if any further information is required, the Examiner is invited to contact the undersigned at (617) 248-4793. Additionally, please charge any fees that may be required, or credit any overpayment, to our Deposit Account No. 03-1721.

Respectfully submitted,

Charles Lyon, Ph.D.
Agent for Applicant
Limited Recognition Under 37 CFR §10.9(b)

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Dated: April 2, 2003

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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

All pending claims have been cancelled and new claims 99-119 have been added.

APPENDIX B

CLAIMS PENDING AFTER ENTRANCE OF AMENDMENT

99. A nucleic acid encoding a chimeric transcription factor that comprises:

a transcriptional regulatory domain; and

a nucleic acid binding domain containing at least two nucleic acid binding motifs, which do not occur together in the same protein in nature in the same order, spacing or arrangement as is present in the chimeric transcription factor, wherein:

at least one of the nucleic acid binding motifs comprises a zinc finger; and

the chimeric transcription factor recognizes a nucleic acid sequence not recognized by a protein containing only one of the nucleic acid binding motifs present in the chimeric transcription factor.

100. A nucleic acid encoding a non-naturally-occurring transcriptional regulatory protein that comprises:

a chimeric nucleic acid binding domain including at least two nucleic acid binding motifs, at least one of which is a zinc finger; and

a transcriptional regulatory domain, wherein the non-naturally-occurring transcriptional regulatory protein (a) recognizes a nucleic acid sequence not recognized by a protein containing only one of the nucleic acid binding motifs present in the transcriptional regulatory protein, and (b) when bound to the recognized sequence, regulates transcription from an operatively linked promoter.

101. The nucleic acid of claim 99 or 100, wherein the nucleic acid binding domain includes at least two zinc fingers.

102. The nucleic acid of claim 99 or 100, wherein the nucleic acid binding domain includes at least a second nucleic acid binding motif selected from the group consisting of helix-loop-helix motifs, helix-turn-helix motifs, basic domains, zinc fingers, and combinations thereof.

103. The nucleic acid of claim 99 or 100, wherein the transcriptional regulatory domain activates transcription.

104. The nucleic acid of claim 99 or 100, wherein the transcriptional regulatory domain represses transcription.

105. The nucleic acid of claim 99 or 100, wherein at least one nucleic acid binding motif is selected from the group consisting of helix-loop-helix motifs, helix-turn-helix motifs, basic regions, and combinations thereof.

106. The nucleic acid of claim 99 or 100, wherein the zinc finger is from a protein selected from the group consisting of transcription factor IIIA, SW15, Krüppel, Hunchback, and a steroid receptor.

107. The nucleic acid of claim 99 or 100, wherein the zinc finger is from Zif268.

108. The nucleic acid of claim 99 or 100, wherein the at least two nucleic acid binding motifs are separated by at least one amino acid.

109. The nucleic acid of claim 99 or 100, wherein each of the nucleic acid binding motifs, when incorporated into a protein, binds to a specific DNA sequence element.

110. The nucleic acid of claim 109, wherein the nucleic acid encodes a protein that recognizes a composite binding site made up of the specific DNA sequence elements recognized by the nucleic acid binding motifs.

111. The nucleic acid of claim 110, wherein the nucleic acid encodes a protein that binds to the composite binding site with higher affinity than it does to any of the specific DNA sequence

elements.

112. A vector comprising a nucleic acid of claim 99.

113. The vector of claim 112, further comprising expression control sequences permitting gene expression in eukaryotic cells.

114. A kit comprising a vector of claim 112 and a gene operably linked to a composite binding site to which the chimeric transcription factor encoded by the vector binds.

115. A vector comprising a nucleic acid of claim 100.

116. The vector of claim 115, further comprising expression control sequences permitting gene expression in eukaryotic cells.

117. A kit comprising a vector of claim 115 and a gene operably linked to a composite binding site to which the non-naturally-occurring transcriptional regulatory protein encoded by the vector binds.

118. A method for modulating expression of a gene in a cell, comprising:

providing a cell containing a chimeric DNA binding element operatively linked to a promoter; and

expressing the nucleic acid of claim 99 in the cell, such that the chimeric transcription factor is produced, binds to the chimeric DNA binding element, and regulates transcription from the promoter.

119. A method for modulating expression of a gene in a cell, comprising:

providing a cell containing a chimeric DNA binding element operatively linked to a promoter; and

expressing the nucleic acid of claim 100 in the cell, such that the non-naturally-occurring transcriptional regulatory protein is produced, binds to the chimeric DNA binding element, and regulates transcription from the promoter.